



ORIGINAL RESEARCH ARTICLE

DELIGNIFICATION TRENDS IN PRETREATMENT OF *Musa paradisiaca* PSEUDO-STEM  
AND THE PRODUCTION OF BIOETHANOL

F. K. Owofadeju, T. E. Kolajo\* and O. A. Onibudo

Department of Wood Products Engineering, University of Ibadan, Ibadan, Nigeria

\*Corresponding author's email address: [tolukolajo@yahoo.com](mailto:tolukolajo@yahoo.com)

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ABSTRACT

This study was conducted to assess the potential of pre-treated *Musa paradisiaca* pseudo-stem as an alternative source of raw material for ethanol production. The pseudo-stem was pre-treated with NaOH and NaOH-AQ at varying time and liquor concentrations using a digester. Cellulose yield (CY) was determined gravimetrically, Residual Klason Lignin (RKL) was evaluated in accordance with TAPPI T236 standards and the effect of the pre-treatment variables were examined. Acid hydrolysis using dilute solutions of HCL and H<sub>2</sub>SO<sub>4</sub> followed pre-treatment, while fermentation was achieved by the addition of an active yeast strain (*Saccharomyces cerevisiae*). The ethanol produced was distilled, qualitative analysis was conducted using the Fourier Transform Infrared Spectroscopy (FTIR) and other standard methods. There was a general decrease in CY and RKL with increasing pulping time and alkali charge, using both NaOH and NaOH-AQ. CY ranged from 2.4 – 4.2g and 3.2 - 5.0g while RKL ranged from 2.46%-3.9% and 1.94-3.00% for both NaOH and NaOH-AQ pre-treatments respectively. However, there was significant difference between the effects of liquor concentration and time on CY at p=0.05. The addition of 0.1% anthraquinone to soda pulping liquor significantly increased CY and decreased RKL. The difference in glucose yields between the two dilute acid hydrolyses was not significant. Various qualitative tests on bio-ethanol showed traits very similar to the laboratory grade ethanol while FTIR confirmed the presence of ethanol. It is therefore proven that NaOH and NaOH-AQ are suitable pretreatment methods in the production of bioethanol from *Musa paradisiaca*.

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1.0 Introduction

Biomass utilization in the production of bioenergy provides an alternative to the use of fossil fuels and, as a result, reduces greenhouse gas emissions and restoring ecosystem balance (Ben Iwo et al., 2016). Biomass for bioenergy also alleviates concerns about the depletion of nonrenewable petroleum resources used in the production of fuels, chemicals, and other bio-products. A rational approach to the use of agricultural and forestry residues has also been necessary.

First generation biofuels were created by fermenting the sugar found in grain starches like corn, sorghum, cassava, and barley as well as the sugar in sugar cane and beets (Alalwan et al., 2019; Dahman et al., 2019) as evidenced by the world's top two producers of ethanol, United States and Brazil, which use corn and sugarcane respectively to produce about three-quarters of the world's ethanol (Yesmin et al., 2020; Fu et al., 2022). This has led to competition between the economies of food and energy. Second generation biofuels investigate the hydrolysis and fermentation of lignocellulose carbohydrates (Baruah et al., 2018; Garba 2021) using feedstocks

such as corn stalks, rice stalks, plantain pseudo-stems, banana pseudo-stems, poplar and willow trees, grasses such as hemp and switchgrass, capable of producing two rounds of harvests per year continuously without the need for replanting, and biomass found in municipal wastes (USEIA, 2022).

The plantain pseudo-stem has been identified as a biomass that can be used for a variety of purposes, including bioethanol production (Ahmad and Danish, 2018). It is the leftover plantain bunch material that has been harvested and allowed to rot. This feedstock is cheap, plentiful, and renewable. The presence of lignin between and within the cell-wall of cellulose microfibrils, which results in recalcitrance during hydrolysis, makes it, like other biomass feedstocks, difficult to process. To hydrolyze and ferment lignocellulose sugars into ethanol, lignin must be eliminated (Islam *et al.*, 2019).

The optimization of ethanol production processes from lignocellulosic biomass is regarded as important research from both an industrial and a scientific standpoint (Ramaraj and Unpaprom, 2019). One of the primary reason bioethanol has struggled to compete favorably with fossil fuels is the lack of a commercially viable manufacturing cost. This begins with the selection of appropriate pretreatment methods to overcome lignin's recalcitrance. The presence of lignin in lignocelluloses has hampered bioethanol production (Zhao and Zhao, 2018). For the purpose of removing lignin from the cellulose matrix, a number of pretreatment procedures have been investigated, including diluted acid pretreatment (Li *et al.*, 2016; Thomas *et al.*, 2017; Liu *et al.*, 2018), ultrasound pretreatment (Ivetic *et al.*, 2017; Luzzi *et al.*, 2017), ionic liquids (Liu *et al.*, 2016; Raj *et al.*, 2018), Since biomass differs in morphology, chemical composition, and structure, a suitable, appropriate, and commercially viable pretreatment method for each specific biomass is required.

In this study, plantain pseudo-stems were pre-treated with soda (NaOH) and soda/antraquinone (NaOH-AQ) before being hydrolyzed and fermented to produce bioethanol. The pretreatment variables' effects on cellulose yield and residual lignin were investigated and a t-test was conducted to ascertain the significant levels of the differences.

## **2.0 Materials and Methods**

### **2.1 Collection and Preparation**

Pseudo stems from a mini-plantation was harvested at the University of Ibadan in Nigeria, located at latitude 7.44°N and longitude 3.90°E. The pseudo-stems were collected, and the leaf was identified in the University of Ibadan's Herbarium as belonging to the *Plantae* kingdom, *Musaceae* family, *Musa* genus and *paradisica* species. For easier pulping, the stems were cut into 3-5 cm chips.

### **2.2 Pretreatment**

Dissolution and serial dilutions of sodium hydroxide pellets with de-ionized water were used to prepare the NaOH pulping liquor while 0.1% anthraquinone were combined with sodium hydroxide to create NaOH-AQ liquor. The samples were pretreated in a 6-liter chemical reactor

built for low temperature biomass pretreatments (Kolajo and Onilude, 2016). The biomass to liquor ratio was 1 to 15 w/v while a temperature of 120°C, was maintained throughout the pulping experiment as adopted by Kolajo (2021). Pulping duration was varied (30, 45 and 60 minutes) at 10-, 12-, and 14% alkali concentrations. The resulting cellulose substrate was washed with distilled water before being refined in a lab refiner for a minute and then put through a 0.1mm sieve. The pulp yield was measured gravimetrically following electronic oven drying to a constant weight.

### 2.3 Dilute Acid Hydrolyses

Separately, 0.5g of cellulose substrate was mixed with 50 ml of 0.2M HCL and H<sub>2</sub>SO<sub>4</sub> in accordance with Camargo et al. (2019). The reaction was run at 25°C for two hours. The reaction was stopped by neutralizing the acid while additionally changing the pH by adding 25ml of concentrated potassium hydroxide solution. The resulting glucose mixture was filtered to remove impurities and then fermented.

### 2.4 Fermentation and Distillation

Fermentation was performed at 37°C. The experiment was conducted in a DNP-9052-1A Thermostat Incubator. A 500ml glucose solution made from HCL and H<sub>2</sub>SO<sub>4</sub> hydrolyzed cellulose substrates was mixed with 1.0g of an active yeast strain (*Saccharomyces cerevisiae*). The yeast was mixed evenly, the mixture was labeled, and it was placed in the incubator for 48 hours (Kolajo, 2021). The resulting ethanol was distilled at 78°C using a simple batch distillation method according to ASTM D86.

### 2.5 Qualitative Tests

For the qualitative analysis, the FTIR device (Buck Scientific Infrared Spectrometer M350) was used (Gallignani et al., 1994; Conklin et al., 2014). A Buck Scientific Infrared Spectrometer with the model number M530 was used to gather the infrared (IR) spectra of the solutions over a 500–4000 cm<sup>-1</sup> range. The absorbed radiation was transformed into rotational and/or vibrational energy by the sample molecules. The resulting signal, which was displayed as a spectrum at the detector typically ranges from 400 to 4000 cm<sup>-1</sup>, representing the molecular fingerprint of the sample. The samples were compared to the pure ethanol's infrared spectra, which were obtained. The ASTM D7795-12 standard was also used for chemical tests (acidity and alkalinity) as well as physical tests (density, boiling point, odor, and color).

## 3.0 Results and Discussion

### 3.1 Cellulose Yield of Pretreated Plantain Pseudo-stem

The highest yield obtained for the plantain pseudo-stem using only NaOH and NaOH-AQ pretreatments was 4.2g at 10% concentration and 30 minutes, and 5.1g at 10% concentration and 30 minutes. Tables 1 and 2 show that the yield for both NaOH and NaOH-AQ pulping was in the range of 2.4 - 4.2g and 3.2 - 5.0g, respectively. The cellulose yield difference between NaOH and NaOH-AQ pulping is significant at p-value of 0.05, indicating that the addition of AQ to NaOH pretreatment significantly yielded a higher cellulose volume for hydrolysis, as also reported by Khristova et al. (2006) and Lois-Correa (2012).

### 3.2 Residual Klason Lignin (RKL) of Pretreated Plantain Pseudo-stem

For the NaOH and NaOH-AQ pulping of the samples, the RKL of the pre-treated biomass ranged from 2.46 to 3.9% and 1.94 to 3%, respectively (Tables 1 and 2). For the NaOH and NaOH-AQ pulping of the samples, the pretreatment with the lowest RKL was 2.46 and 1.94%; this indicates an average of over 60% lignin removal in both schedules. However, according to Xu *et al.* (2011), the amount of RKL is within the range that permits cellulose hydrolysis. The results of the analysis of variance reveal a significant difference between the RKL values of pulping with NaOH and NaOH-AQ.

**Table 1:** Pretreatment Variables and Pulp Yields using NaOH

Conc. (%)	Time (mins)	Yield (g)	Kappa No.	RKL
10	30	4.27 ± 0.06	30.17	3.900
10	45	4.03 ± 0.15	29.00	3.770
10	60	3.17 ± 0.15	21.10	2.743
12	30	3.80 ± 0.10	26.10	3.393
12	45	3.67 ± 0.21	21.40	2.782
12	60	3.07 ± 0.06	18.90	2.457
14	30	3.47 ± 0.15	23.40	3.042
14	45	2.70 ± 0.10	20.10	2.623
14	60	2.47 ± 0.06	18.90	2.457

**Table 2:** Pretreatment variables and Pulp Yields using NaOH-AQ

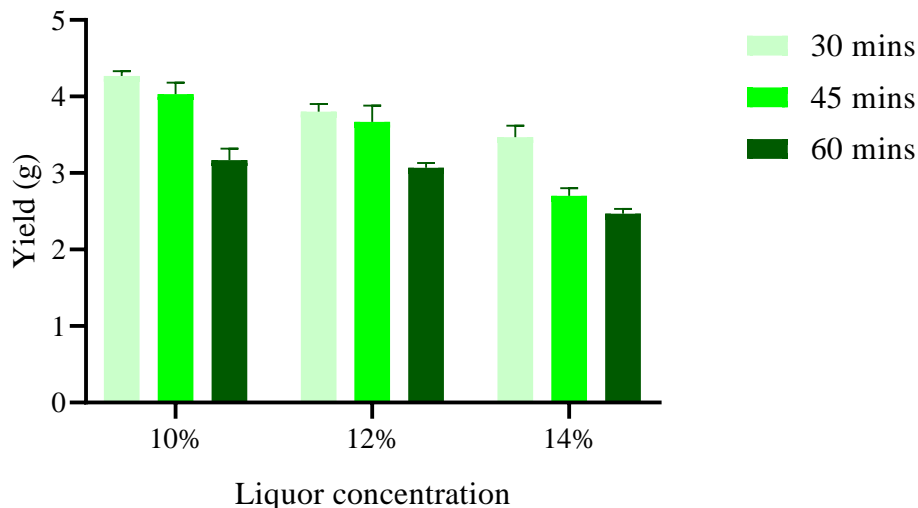
Conc. (%)	Time (mins)	Yield (g)	Kappa No.	RKL
10	30	5.13 ± 0.15	23.10	3.003
10	45	4.93 ± 0.12	22.20	2.886
10	60	4.37 ± 0.06	19.20	2.496
12	30	4.53 ± 0.06	22.20	2.886
12	45	4.37 ± 0.15	18.90	2.457
12	60	4.00 ± 0.10	15.90	2.067
14	30	3.83 ± 0.15	19.20	2.496
14	45	3.57 ± 0.06	16.50	2.145
14	60	3.234 ± 0.15	14.90	1.937

### 3.3 Effect of Pretreatment on Cellulose Yield and RKL

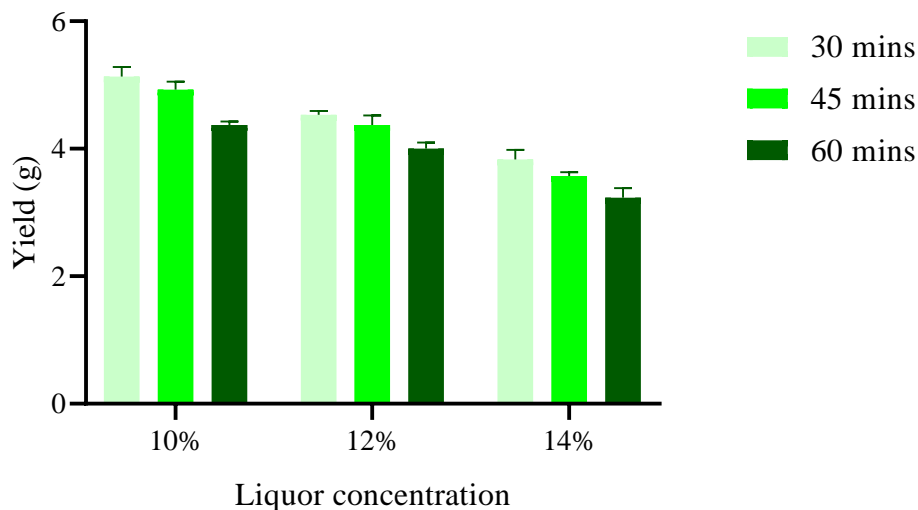
#### 3.3.1 Effect of Pretreatment Time on Cellulose Yield

The effects of pretreatment time on the yield of pre-treated plantain pseudo-stem for NaOH and NaOH-AQ are depicted in Figures 1 and 2. As the cooking time and alkali concentration increased, the cellulose yield decreased. Kolajo and Onilude (2019) speculate that this drop-in yield may be caused by the biomass's extractives and other non-fibrous components dissolving. As a pseudo-stem, there are a number of other cell types present besides cellulose fibres (Patel

and Patel, 2022; Kolajo et al., 2023) which are likely to be dissolved by the cooking liquor. The yield from the pretreatment may be impacted as the carbohydrate content of the biomass dissolves over time.



**Figure. 1:** The effect of pretreatment time on Cellulose yield using NaOH



**Figure 2:** The effect of pretreatment time on Cellulose yield using NaOH-AQ

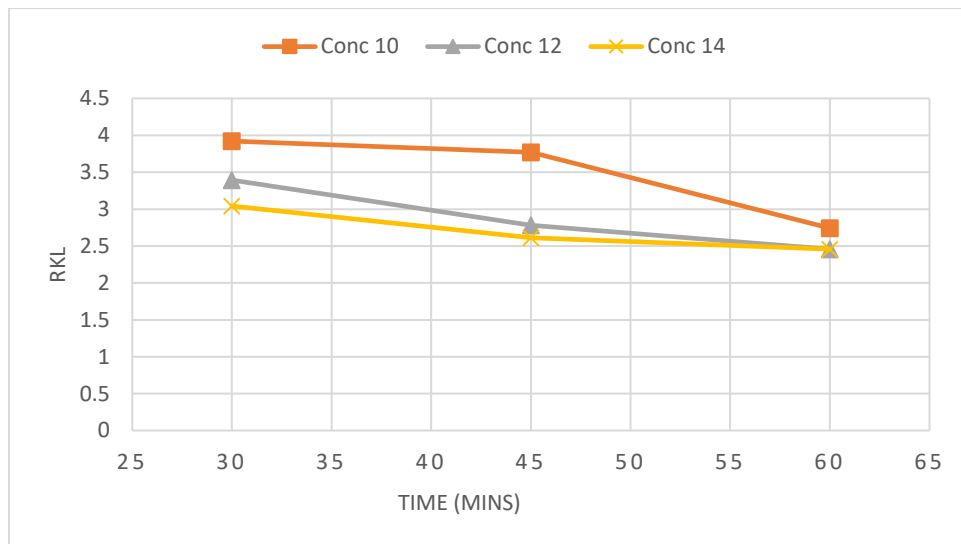
### 3.3.2 Effect of Liquor Concentration on Cellulose Yield

An increase in alkali charge resulted in a decrease in the cellulose yield obtained from pretreatment at constant temperature and pretreatment duration. This pattern can be seen in the pulping of the plantain pseudo-stem with NaOH and NaOH-AQ (Figures 1 and 2). The increase in alkali charge indicated by the graphs may be the cause of this. Because the alkali affects both the lignin and carbohydrate components of the pseudo stem, an increase in alkali charge led to a corresponding decrease in yields. These observations correlate with other findings in

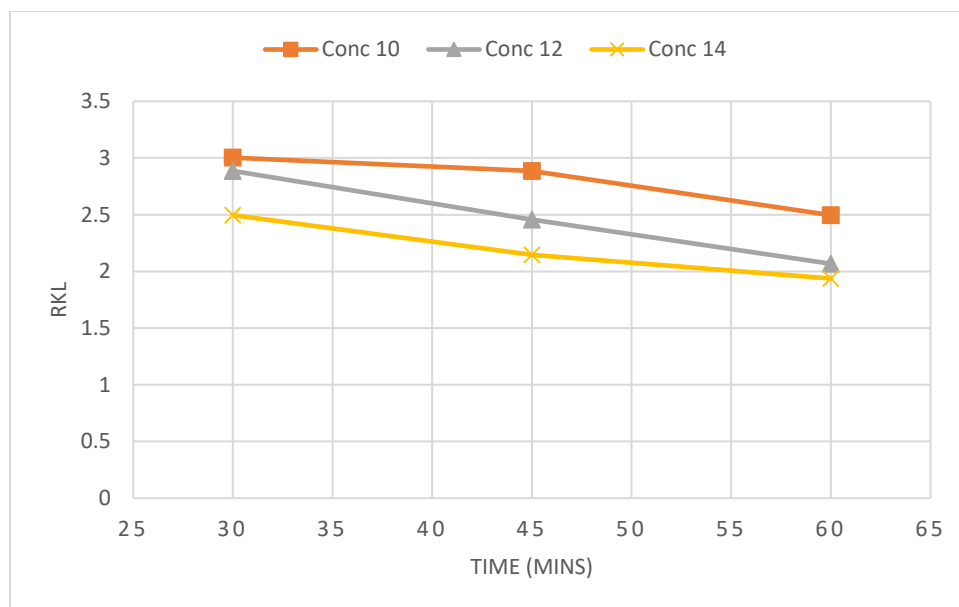
literature (Jie *et al.*, 2022, Kolajo 2021) who reported that total pulp yield is decreased as active alkali charge is increased.

### 3.3.3 Effect of Time and Liquor Concentration on RKL

Figures 3 and 4 show how RKL is impacted by both time and alcohol concentration. As the time was extended, the lignin content further decreased, resulting in a lower Kappa number and RKL. A rapid decrease in RKL was observed between 45 to 60 minutes of pretreatment at 10% concentration in contrast to 12 and 14% alkaline charges. This trend was similar in both NaOH and NaOH-AQ pretreatments. This may be attributed to the dissolution of the non-fibrous components of the biomass first, before delignification. This then implies that pulping at 10% alkali charge will require a longer duration for delignification to occur. However, RKL across the liquor concentrations also decreased as the time was increased, indicating lignin dissolution.



**Figure 3:** Effect of Time and NaOH Concentration on RKL of Plantain Pseudo-stem



**Figure 4:** Effect of Time and NaOH-AQ Concentration on RKL of Plantain Pseudo-stem

### 3.4 Qualitative Tests

The bioethanol produced is a clear, colorless liquid that boiled between 79 and 82°C. The tested density varied from 0.83g/mL to 0.85g/mL. After distillation at between 80 and 82°C, there was complete miscibility with water and no residue was left behind. The pH ranged from 7.5 to 8.0. The test results supported the presence of ethanol as reported by Suleiman *et al.* (2017) and Kolajo (2021) when compared to laboratory grade ethanol (Analar® chemicals).

### 3.5 Fourier Transform Infrared Spectroscopy Analysis (FTIR)

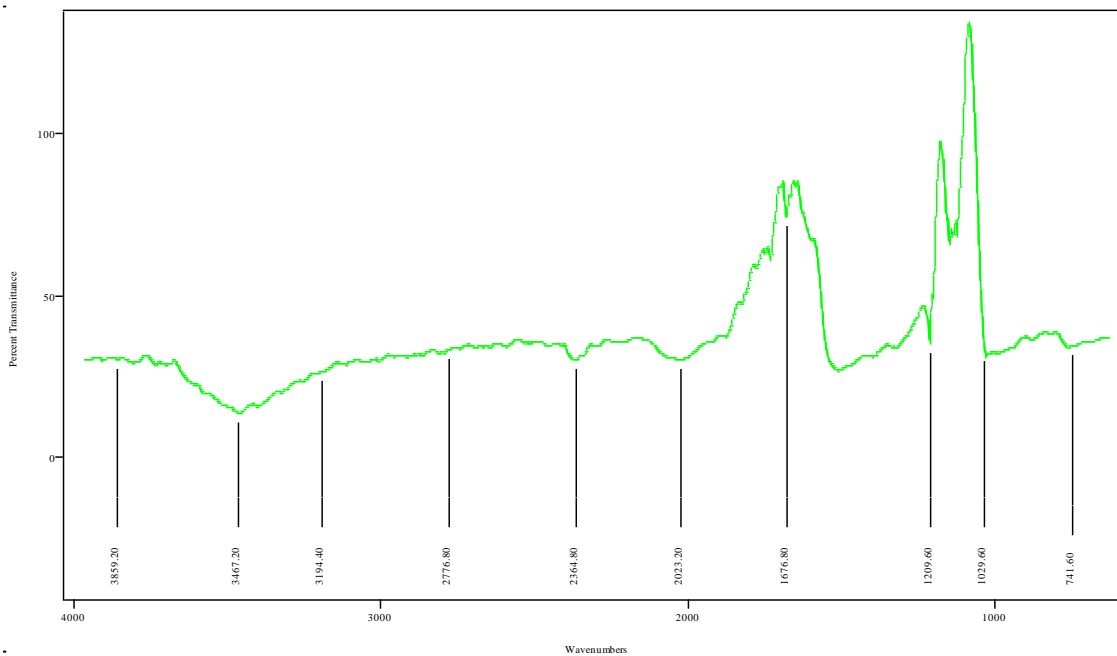
The results of the FTIR analysis of the bioethanol made from plantain stem biomass are shown in Table 3. The vibrational frequencies for (O-H), (C-H), and (C-O) for sample A, as depicted in Figure 5, were noted at 3467  $\text{cm}^{-1}$ , 2780  $\text{cm}^{-1}$ , and 1209  $\text{cm}^{-1}$ , respectively. Figure 6 illustrates the vibrational frequencies for (O-H), (C-H), and (C-O) for sample B at 3189  $\text{cm}^{-1}$ , 2775  $\text{cm}^{-1}$ , and 1072  $\text{cm}^{-1}$ .

**Table 3.** The Infrared Spectra of Bioethanol Obtained from Plantain Pseudo Stem ( $\text{cm}^{-1}$ )

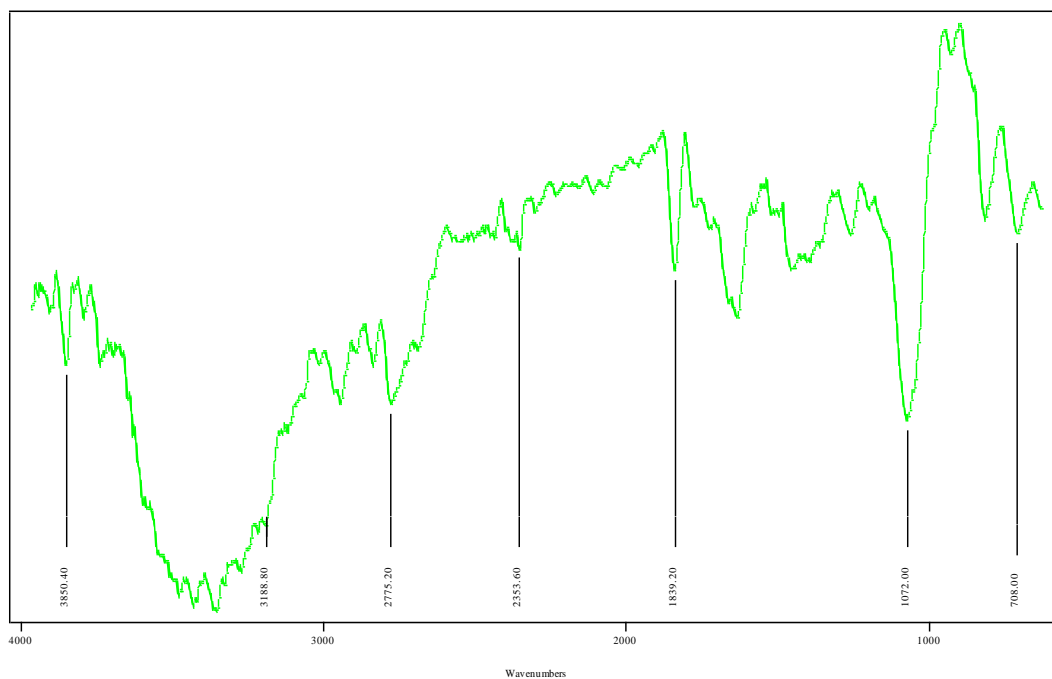
Sample code	Acids	O-H	C-H	C-O
A	HCl	3467	2780	1209
B	H <sub>2</sub> SO <sub>4</sub>	3189	2775	1072

All produced bioethanol spectra from the hydrolysis of both hydrochloric and sulphuric acids contained bands in the 3189–3467  $\text{cm}^{-1}$  range for the hydroxyl functional group (OH) (Forough *et al.*, 2013). While the stretching vibrations of (C-O) were responsible for the absorbance peaks around 1072–1209  $\text{cm}^{-1}$ , the stretching vibrations of (C-H) were responsible for the absorbance peaks at 2775–2780  $\text{cm}^{-1}$ . The samples' similar bands served as proof that they were all bioethanol samples. Numerous functional groups, including hydroxyl (OH stretch), methyl (CH<sub>3</sub> stretch),

and alkane ( $\text{CH}_2$  stretch), are present in bioethanol. The infrared spectra of bioethanol show transmittance and a peak corresponding to a functional group. These functional groups can also be seen in the spectra of pure ethanol (Ogidi, 2020). In the (O-H) stretching region between  $3000 \text{ cm}^{-1}$  and  $3600 \text{ cm}^{-1}$  are reported to have distinct broad bands in the infrared spectra of pure ethanol and blends, according to Corsetti *et al.* (2015). Additional ethanol-specific traits can be found in the fingerprint region.



**Figure 5:** FTIR for Hydrolyzed Substrate using HCl



**Figure 6:** FTIR for Hydrolyzed Substrate using  $\text{H}_2\text{SO}_4$



### 3.6 Conclusion

There is a general decrease in cellulose yield and residual klason lignin as pretreatment time and concentration increased in both soda and soda anthraquinone pulping processes. This trend also confirmed that lignin condensation did not occur. Cellulose yield is increased significantly with the addition of 0.1% anthraquinone to soda pulping liquor with a higher decrease in Residual Klason Lignin. There was sufficient delignification as confirmed by the subsequent hydrolysis and fermentation into ethanol. The properties of bioethanol produced compared well with laboratory grade ethanol. This has established that plantain pseudo-stem wastes can be utilized for bioethanol production using low temperature and cost-effective pretreatments that can be explored for biomass having similar morphology.

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